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Sex Reversal in C57BL/6J-Y<latex>$^{\text{POS}}$</latex> Mice Corrected by a Sry Transgene [and Discussion]

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Sex reversal in C57BL/6J-Y^{POS} mice corrected by a *Sry* transgene

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SUMMARY

C57BL/6J mice carrying a *Mus domesticus poschiavinus* Y chromosome (Y^{POS}) develop as females with ovarian tissue or as hermaphrodites with ovarian and testicular tissue. We tested the hypothesis that the Y-linked component of this inherited sex reversal is caused by the *M. d. poschiavinus* Y-linked testis determining gene (symbolized *Tdy* or *Sry*) by examining gonadal development in C57BL/6J XY^{POS} mice carrying a *M. musculus* allele of *Sry* as a transgene. We found that in the presence of the transgene, XY^{POS} mice developed exclusively testicular tissue. This result indicates that the *Sry* allele carried on the Y^{POS} chromosome is responsible for development of ovarian tissue in the C57BL/6J inbred strain background. We discuss this finding in light of DNA polymorphisms present in *Sry* alleles carried by various *M. domesticus* and *M. musculus* Y chromosomes. In addition, we present a hypothesis concerning the timing of expression of the testicular and ovarian determining genes in the developing fetal gonad based on the organization of ovarian and testicular tissue in ovotestes.

1. INTRODUCTION

In 1982 we described an inherited condition in mice in which XY individuals developed ovarian tisue (Eicher *et al.* 1982). This sex reversal condition was identified during the transfer of the Y chromosome from mice of the subspecies *Mus domesticus poschiavinus* (POS) onto the C57BL/6J inbred strain background using a standard backcrossing method. After a few generations of backcrossing, half of the XY individuals developed as females with ovarian tissue and half developed as hermaphrodites (individuals containing both ovarian and testicular tissue); no XY individual developed exclusively testicular tissue. Some hermaphrodites breed as males if they develop sufficient testicular tissue to masculinize their internal and external genitalia and produce sufficient numbers of sperm to fertilize eggs. These fertile hermaphrodites allow us to maintain the C57BL/6J-Y^{POS} strain and perform genetic experiments, which otherwise would be impossible because XY^{POS} females are sterile.

The inability of the *M. d. poschiavinus* Y chromosome to cause normal testicular development when placed onto the C57BL/6J inbred strain is not unique. For example, four other *M. domesticus* Y chromosomes, each derived from wild mice trapped in different regions of Europe, cause ovarian tissue development when placed on the C57BL/6J background (Eicher *et al.* 1982; Nagamine *et al.* 1987). Other *M. domesticus*-derived Y chromosomes, however, result in normal testicular development when placed on C57BL/6J. Examples include the Y chromosomes carried by standard inbred strains (e.g. AKR/J, SWR/J, SJL/J, and BUB/J) as well as several Y chromosomes obtained fom wild males trapped in Europe and North America (E. Eicher, unpublished data).

Further investigation indicated that when C57BL/6J XY^{POS} hermaphrodites are mated to DBA/2J, C3H/HeJ, or BALB/cBy females, the XY F1 mice develop exclusively testicular tissue (Eicher & Washburn 1986). When these F1 males are backcrossed to C57BL/6J females, however, some XY backcross offspring develop as hermaphrodites, with the frequency of hermaphroditism dependent on the specific inbred strain used (E. Eicher, unpublished data).

The simplest hypothesis to explain C57BL/6J-Y^{POS} inherited sex reversal is that an abnormal interaction occurs between the *Sry* allele carried on the *M. d. poschiavinus* Y chromosome and one or more C57BL/6J-derived alleles carried on autosomes and possibly the X chromosome (Eicher *et al.* 1982). Some investigators have regarded the Y-linked portion of this hypothesis as proven and have used DNA sequence data obtained from different *Sry* alleles to suggest which differences account for the abnormal behavior of the Y^{POS} chromosome (Coward *et al.* 1994). This interpretation, however, was premature because the mouse Y chromosome contains many genes (Bishop 1992). Thus, it is possible that a Y-linked gene other than *Sry* is responsible for the sex reversal condition in C57BL/6J XY^{POS} mice.

Here we describe results from experiments designed to test the hypothesis that *Sry* is the gene on the *M. d. poschiavinus* Y chromosome responsible for ovarian tissue development in C57BL/6J XY^{POS} mice. The experimental system we employed is similar to a conventional genetic complementation test except that one of the alleles, the *Sry* allele from *M. d. poschiavinus* (*Sry*^{POS}), is present in its normal (endogenous) position on the Y chromosome and the other allele, the *Sry* allele from the 129 inbred strain (*Sry*^{129}), is inserted as a transgene into an ectopic position in the genome. The

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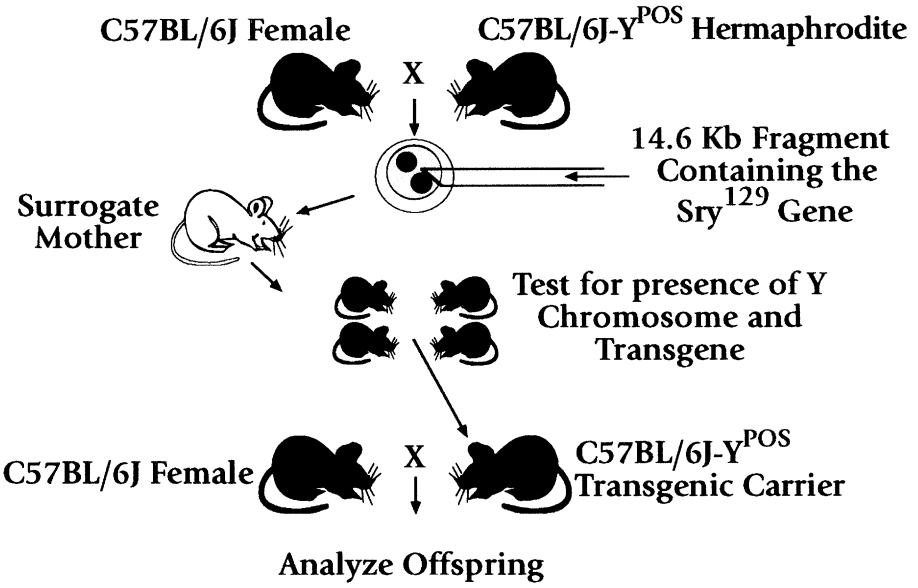

Figure 1. Scheme for producing C57BL/6J XY^{POS} mice carrying the *Sry*^{129} allele as a transgene.

transgene used was a 14.6 k.b. DNA fragment isolated from the Y chromosome of the 129 inbred strain (Gubbay *et al.* 1990). We chose this DNA fragment because: (i) transfer of the 129 Y chromosome (*M. musculus* origin) onto the C57BL/6J strain does not result in sex reversal (unpublished data); (ii) *Sry* is the only gene present in this DNA fragment (Gubbay *et al.* 1992), thus restricting the complementation test to the *Sry* gene; and (iii) transgenic XX mice carrying this DNA fragment can develop testes, indicating that all of the Y chromosome sequences necessary to initiate testicular development in the developing fetal gonad are present, including cis-acting regulatory sequences (Koopman *et al.* 1991).

2. PRODUCTION OF TRANSGENIC MICE

Transgenic mice were produced using standard methods (Wagner *et al.* 1981). The 14.6 k.b. DNA fragment containing the *Sry*^{129} gene was injected into the male pronucleus in fertilized eggs obtained from C57BL/6J females mated to C57BL/6J XY^{POS} hermaphrodites. The breeding scheme used to produce the transgenic mice is shown in figure 1. Two polymerase chain reaction (PCR) assays (see figure 2) were developed to distinguish the *Sry*^{129} and *Sry*^{POS} alleles and to independently determine the presence of a Y chromosome. Of the potential transgenic mice typed, two XY individuals contained a transgene. One of the transgenic mice was a sterile hermaphrodite and he was not analysed further. The second transgenic mouse was a fertile male and he and his offspring are the subject of this paper.

3. ANALYSIS OF TRANSGENIC MALE AND HIS OFFSPRING

At two weeks-of-age the transgenic male was notable because his external genitalia were that of a normal male and he lacked mammary-associated yellow pigmented hairs, a condition present in all XX and XY females and many hermaphrodites of the C57BL/6J-Y^{POS} strain. To determine whether he would transmit the transgene to his offspring, we mated him to C57BL/6J females and analysed the offspring for their sex chromosome constitution, external genitalia, and presence of the transgene. Approximately 24% (11 of 46) of his offspring inherited a transgene. However, the inheritance pattern was somewhat complicated because two separate transgene insertions were detected: a low copy number insertion that did not affect sexual development in XX transgenic offspring and a high copy number insertion that caused male development in XX transgenic offspring. Southern blot analysis indicated that the insertion site for each transgene was independent (data not shown). Each transgenic offspring inherited either one or the other transgene and each transgene was inherited by both XX and XY offspring. We conclude from these results that each transgene insertion was present in a different germ cell population and that both transgene insertions were into an autosome. Official designations for the high and low copy number transgenes are *Tg*N(*Sry*)*4Ei* and *Tg*N(*Sry*)*5Ei*, respectively.

To determine if *Tg*N(*Sry*)*4Ei* (*Tg4*) was responsible for the correction of the gonadal defect in C57BL/6J XY^{POS} mice, an XY^{POS} transgenic son and two XY^{POS} transgenic grandsons from the transgenic founder were mated to C57BL/6J females. Fetal offspring were analysed at 14.5–16 days of embryonic development for gonadal development and sex chromosome constitution. This developmental period was chosen because a small amount of one type of gonadal tissue is easily observed when present with a majority of the other type of gonadal tissue, whereas later in development a minority of ovarian tissue can be obscured (Eicher *et al.* 1980). As expected, *Tg4* segregated as a Mendelian trait (see table 1). In addition, all XX fetuses lacking *Tg4* developed ovarian tissue, and all XY fetuses lacking *Tg4* developed either ovarian

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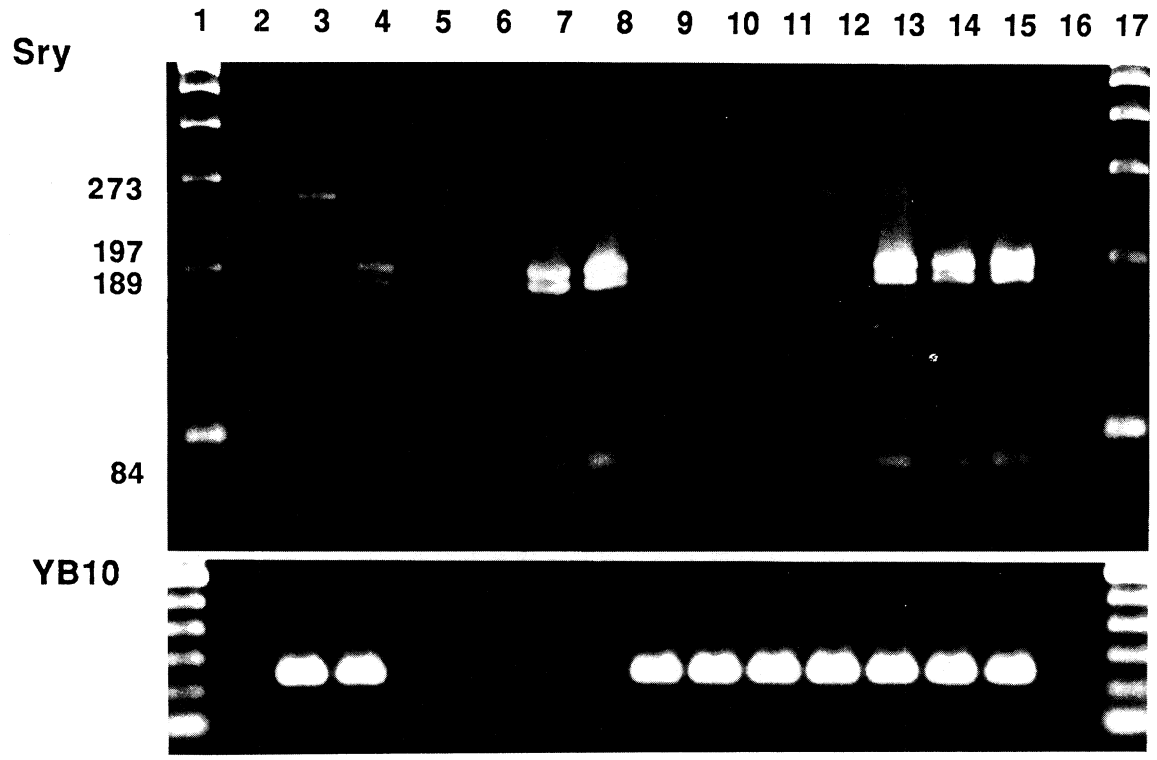

Figure 2. Determination of the presence of *Tg4* and the Y chromosome. Top, presence of the transgene was determined by PCR amplification of a 470 b.p. segment from the *Sry* gene using the primer pair: 5′-AGATCTTGATTTTTA-GTGTTC-3′; 5′-GAGTACAGGTGTGCAGCTCTA-3′) (Gubbay *et al.* 1990). PCR products were cut with *Mbo*I, and the resulting fragments separated by size on a 4% NuSieve 3:1 agarose gel. Two fragments (273 and 197 b.p.) are diagnostic for *Sry^{POS}* and three fragments (197, 189, and 84 b.p.) are diagnostic for *Sry^{129}* (the 129 PCR product contains an additional *Mbo*I site). Lanes 1–4, 16 and 17 represent control DNA samples: 1 and 17 are molecular size markers, 16 is a no DNA control, 2 is a C57BL/6J XX female, 3 is a C57BL/6J XY^{POS} hermaphrodite, and 4 is a 129/J male. Lanes 5–15 represent DNA samples from C57BL/6J fetuses from the *Tg4* experiment: 5 and 6 are XX females, 7 and 8 are XX *Tg4* males, lane 9 and 10 are XY^{POS} hermaphrodites, 11 and 12 are XY^{POS} females, and 13, 14, and 15 are XY^{POS} *Tg4* males. Bottom, the higher copy number of the *Tg4* transgene made it difficult to determine presence of the endogenous *Sry^{POS}* gene (i.e. the *M. d. poschiavinus* Y chromosome). To circumvent this problem, the presence of the Y^{POS} chromosome was verified by PCR amplification using the primer pair: YB10L, 5′-CCCCTTTCTAAGCAGACATC-3′; and YB10R 5′-TCACACTAACCTCACAGGCC-3′. These primers were derived from a Y chromosome sequence, designated YB10, that is present in 300–500 copies on the long arm of the mouse Y chromosome (Eicher *et al.* 1989; Tucker *et al.* 1989; Tucker *et al.* 1992). The YB10 primers amplify a 225 b.p. fragment from male DNA. Under some conditions, PCR products are noted in female DNA; their sizes, however, differ from the fragment obtained from male DNA. DNA samples are the same as in (*a*) above.

Table 1. *Inheritance of* Tg4 *and gonadal development in offspring from C57BL/6J females mated tp C57BL/6J CY^{POS}* Tg4 *males*

(O and T represents ovary and testis, respectively, and OT represents ovotestis. Analysis was conducted at 14.5–16 days of fetal development.)

	XX	XY^{POS}	XX *Tg4*	XY^{POS} *Tg4*
bilateral gonadal development	O/O	O/O, O/OT, or OT/OT	T/T	T/T
number of fetuses analysed	12	10	7	21

tissue or ovarian and testicular tissues, as is observed in C57BL/6J XY^{POS} mice. Of importance was the gonadal development of the fetuses that inherited *Tg4*. Regardless of their sex chromosome composition, each *Tg4* fetus developed exclusively testicular tissue (see figure 3). From these results we conclude that: (i) *Tg4* is functional because it causes sex reversal when present in XX mice; and (ii) the Y-linked gene responsible for C57BL/6J-Y^{POS} sex reversal is the *Sry^{POS}* gene because *Sry^{129}* is the only gene present in the 14.6 k.b. fragment used to produce the transgenic mice and the presence of the *Sry^{129}* allele corrects the inherited sex reversal defect in the XY^{POS} mice.

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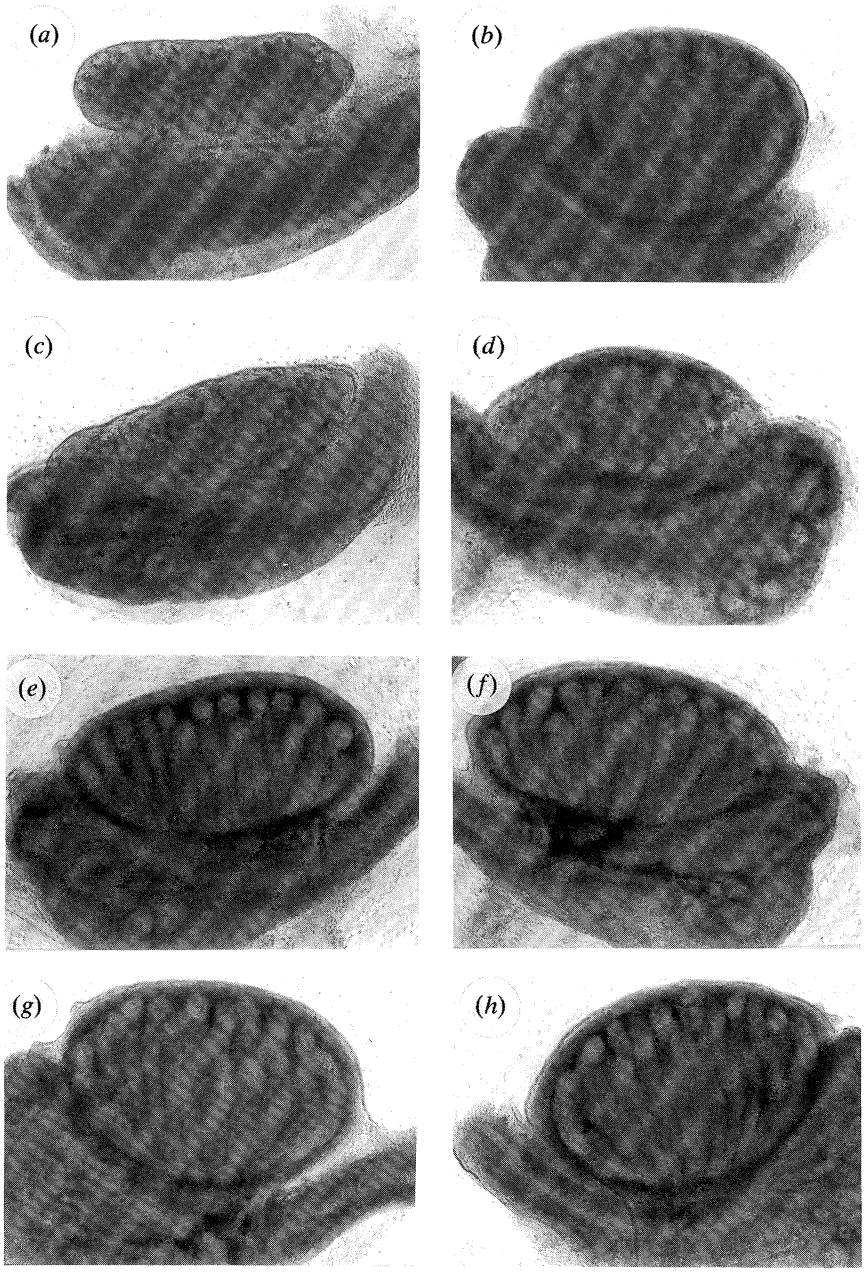

Figure 3. Gonads with attached mesonephric complexes from fetuses at 14.5 days of development. Gonads (*a*) and (*b*) represent an ovary from a normal C57BL/6J XX female and a testis from a normal C57BL/6J XY male, respectively. Gonad (*c*) is an ovary from a C57BL/6J XY^{POS} female and gonad (*d*) is an ovotestis from a C57BL/6J XY^{POS} hermaphrodite. In the ovotestis, note the presence of testicular tissue in the central region with ovarian tissue at both ends. Gonads (*e*) and (*f*) are the paired testes from a C57BL/6J XX *Tg4* male and gonads (*g*) and (*h*) are the paired testes from a C57BL/6J XY^{POS} *Tg4* individual.

3. CONCLUSIONS AND PERSPECTIVES

Experiments in mice using *Sry* as a transgene identified *Sry* as *Tdy* (Koopman *et al.* 1991), the Y-linked gene that was hypothesized to initiate testicular development in XY individuals (symbolized TDF in humans). This information coupled with DNA sequence information that *Sry* encodes a transcription factor (Sinclair *et al.* 1990) suggests *Sry* normally functions by activating one or more genes at the beginning of the testis developmental pathway. We have hypothesized that in parallel to *Sry*, an ovary determining (*Od*) gene carried on an autosome or the X chromosome normally activates the ovarian developmental pathway in XX individuals (Eicher & Washburn 1986). We also hypothesized that, in addition to the role played by *Sry* in activating the testis developmental pathway, *Sry* plays a role in inactivating the ovarian developmental pathway (Eicher & Washburn 1986). In C57BL/6J XY^{POS} mice the *Od* allele is of C57BL/6J (B6) origin and the *Sry* allele is of *M. d. poschiavinus* origin.

We suggest three possibilities that would explain ovarian tissue development in C57BL/6J XY^{POS} mice.

1. The protein encoded by the *Sry^{POS}* gene contains a conformational change (compared to the protein

encoded by the *Sry^{B6}* or *Sry^{129}* alleles) and this change prevents the *Sry^{POS}* protein from forming a stable transcription complex with a C57BL/6J encoded protein.

2. The level of the *Sry^{POS}* protein produced in a C57BL/6J background is not sufficient to activate C57BL/6J testicular genes, or inactivate the C57BL/6J ovarian pathway, or both.

3. The gene that activates *Sry* in a C57BL/6J XY^{POS} mouse is of C57BL/6J origin and this C57BL/6J gene may be unable to activate *Sry^{POS}* sufficiently or in a timely manner, allowing activation of the ovarian pathway.

4. WHAT PART OF THE *SRY^{POS}* GENE IS RESPONSIBLE FOR ABNORMAL TESTICULAR DEVELOPMENT IN C57BL/6J MICE?

It is of interest to determine which DNA sequence(s) within the *Sry^{POS}* gene accounts for its failure to initiate normal testicular development when present in the C57BL/6J genome. Sequence differences have been identified between the *M. musculus Sry* allele carried by the 129 inbred strain and several *M. domesticus Sry* alleles. For example, a C to T transition difference has been noted within the HMG box (Tucker *et al.* 1992). (This *Mbo*I polymorphism was used to detect the two *Sry* alleles typed in the experiments reported here.) The C to T nucleotide transition, however, can not be responsible for development of ovarian tissue in C57BL/6J XY^{POS} mice because all *M. domesticus*-derived *Sry* genes we have tested contain the C to T transition but not all of the Y chromosomes cause sex reversal when placed on the C57BL/6J inbred strain background (unpublished data). In addition, the *Sry* alleles carried by several *M. musculus* Y chromosomes derived from Europe also contain the C to T transition (Tucker *et al.* 1992); these Y chromosomes, however, do not cause sex reversal when placed on C57BL/6J (E. Eicher, unpublished data).

Coward and collaborators have sequenced the open reading frame (ORF) of the *Sry* allele carried on a *M. domesticus* Y chromosome (designated Y^{DOM}) and compared it with the published sequence for the *Sry^{129}* gene (GenBank entry X67204) (Gubbay *et al.* 1992; Coward *et al.* 1994). (The geographic origins of the Y^{DOM} and Y^{POS} chromosomes suggest they are identical and carry the same *Sry* allele. Placement of the Y^{DOM} chromosome onto the C57BL/6J inbred strain background by repeated backcrossing produces a sex reversal condition identical to that observed when the Y^{POS} chromosome is present on the C57BL/6J background (Nagamine *et al.* 1987).) In addition to the C to T difference noted above, other DNA sequence differences were detected between the *Sry^{129}* and *Sry^{DOM}* alleles, including six additional base pair substitutions, five insertions, and the position of the stop codon (reviewed in Eicher 1994). The stop codon in *Sry^{129}* is at position 9489 and results in a predicted protein composed of 395 amino acids. The stop codon in *Sry^{DOM}* is at position 9006 and results in a predicted protein composed of 230 amino acids. Coward and co-workers found that other *M. domesticus*-derived *Sry* alleles, including *Sry^{POS}*, contain the stop codon at the same position as the *Sry^{DOM}* allele. Clearly the presence of a 'premature' stop codon in itself is not responsible for development of ovarian tissue in the C57BL/6J-Y^{POS} mice because *M. d. domesticus Sry* alleles that contain the same stop codon (e.g. SWR/J, SJL/J, AKR/J) fail to cause sex reversal when placed on the C57BL/6J inbred strain background. It is possible that the 'premature' stop codon in *M. domesticus Sry* alleles is responsible for a delay in normal testis formation when it is present in the C57BL/6J genome. Examples of delayed cord formation are observed in males carrying the Y^{POS} and Y^{AKR} chromosomes. Palmer and Burgoyne (Palmer & Burgoyne 1991) and we (E. Eicher, unpublished data) have observed that on genetic backgrounds where the Y^{POS} chromosome does not cause ovarian tissue development, testicular cord formation is delayed. A similar developmental pattern was noted in C57BL/6J (Washburn & Eicher 1983; Washburn & Eicher 1989; Washburn *et al.* 1990) and hybrid (Burgoyne & Palmer 1993) mice carrying an Y^{AKR} chromosome.

Coward and collaborators suggest that the region responsible for the sex reversal condition is at position 8811, where there is a variation in the number of CAG repeats in different *M. domesticus Sry* alleles. We have suggested that the difference in CAG repeats alone is not responsible for ovarian tissue development because the number of CAG repeats at this position only differs by one or two (Eicher 1994). The difference responsible for sex reversal, in fact, may be located outside of the ORF in the *Sry* promoter or another cis-acting regulatory element. Until it is tested directly, however, the CAG repeat hypothesis of Coward and collaborators remains viable.

5. OVARIAN/TESTICULAR TISSUE DISTRIBUTION IN OVOTESTES: INSIGHT INTO THE TIMING OF THE TESTIS AND OVARY DETERMINING GENES?

During our studies of different sex reversal conditions in mice, we have noted that the majority of fetal ovotestes are of two types: those that contain testicular tissue in the central region with ovarian tissue at both ends (see example in figure 3*d*) and those that contain ovarian tissue at one end with testicular tissue occupying the remainder of the gonad. These two types of ovotestes are observed in sex chromosome mosaics (XO/XY or XO/XY/XYY) (Beamer *et al.* 1978; Eicher *et al.* 1980), in opposite sex chimeras (XX↔XY) (E. Eicher, M. P. Rosenberg, and L. L. Washburn, unpublished data), and in the inherited sex reversal conditions C57BL/6J-Y^{POS} and C57BL/6J-*Tas* (Washburn & Eicher 1983; Washburn & Eicher 1989; Washburn *et al.* 1990). Common to all of these sex reversal conditions is the presence of ovarian tissue in those gonadal regions that are the last to form cords in the normal developing testis.

We suggest that the nonrandom organisation of gonadal tissues in ovotestes and the delay in testicular cord formation in some genetic backgrounds provide

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insight into the initial transcription of the *Od* and *Sry* genes. We hypothesize that *Sry* is expressed before *Od* and that *Sry* transcription first occurs in cells situated in the central region of the undifferentiated XY fetal gonad, followed by expression in cells located in the rest of the gonad. In contrast, *Od* expression first occurs in cells located at the ends of the gonad, followed by expression in cells located in the rest of the gonad. A short delay in *Sry* expression would cause a delay in testicular cord formation at the ends of the gonads, as is seen in some mice carrying certain *M. domesticus* Y chromosomes. A more extensive delay in *Sry* expression could overlap initiation of *Od* leading to ovarian tissue development, which is observed in the C57BL/6J XY^{POS} mouse.

We thank Peter Hoppe for making the transgenic mice, Robin Lovell-Badge for sharing the L741, p422.04 and p4.2.2 probes used to distinguish *Tg4* and *Tg5*, and John Gubbay for providing technical advice for isolating the 14.6 k.b. insert from L741. In addition, we thank Kenn Albrecht, Wayne Frankel, and Keith Hutchison for critically reading an earlier version of this manuscript. This research was supported by NIH grant GM20919.

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Discussion

P. Burgoyne (*NIMR, The Ridgeway, Mill Hill, London, U.K.*). M. Ferguson-Smith encouraged Dr Eicher to draw the conclusion that the B6 autosomal genes Dr Eicher is studying must be downstream of *Sry* in the testis-determining pathway. As Dr Eicher knows, I am a champion of the alternative possibility that they may be involved in ovary determination. S. Palmer in my lab has shown that the poschavinus Y chromosome, when assessed on a F1 background, triggers testis cord formation 14 h later than the B6 Y on the same genetic background. The AKR Y is intermediate with respect to timing. Thus the Y chromosomal component of B6Y^{POS} XY sex reversal manifests as a delay in the testis determination pathway. We have therefore proposed a 'timing mismatch' model which suggests that the B6 autosomal alleles Dr Eicher is studying are associated with an earlier ovary determination process than most other inbred strains. This early acting ovary determination is then able to completely pre-empt the late-acting Y^{POS}, thus producing partial or complete XY sex reversal.

E. M. Eicher. The presence of ovarian tissue in C57BL/6J XY^{POS} mice suggest that when the Sry^{POS} gene is present in the C57BL/6J genome, it is unable to 'lock in' the testis determining pathway before the ovarian determining pathway is activated. We have proposed that mammals have developed a system to guarantee testicular tissue development in an XY individual, by activating the testis determining

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(*Tdy* or *Sry*) gene sufficiently earlier than when the ovary determining (*Od*) gene is normally activated. There are two ways that ovarian tissue could form in C57BL/6J XY^{POS} mice if the problem involves the relative timing of the Sry^{POS} and *Od*^{B6} genes: (i) the *Sry*^{POS} gene acts too late relative to the *Od*^{B6} or (ii) the *Od*^{B6} gene acts earlier than the *Sry*^{POS} gene. Either case would shorten the time needed by the *Sry* gene to lock in the testis pathway and prevent the *Od* gene from functioning. One of the autosomal genes we have mapped may be the *Od* gene (thus it is an ovarian pathway). One of these genes may activate *Sry* (thus it is in the testis pathway), and one or all three of these genes may function downstream of *Sry* (thus it is the testis pathway). Until the autosomal genes are cloned and can be biologically manipulated, however, we can argue for any one of these possibilities.

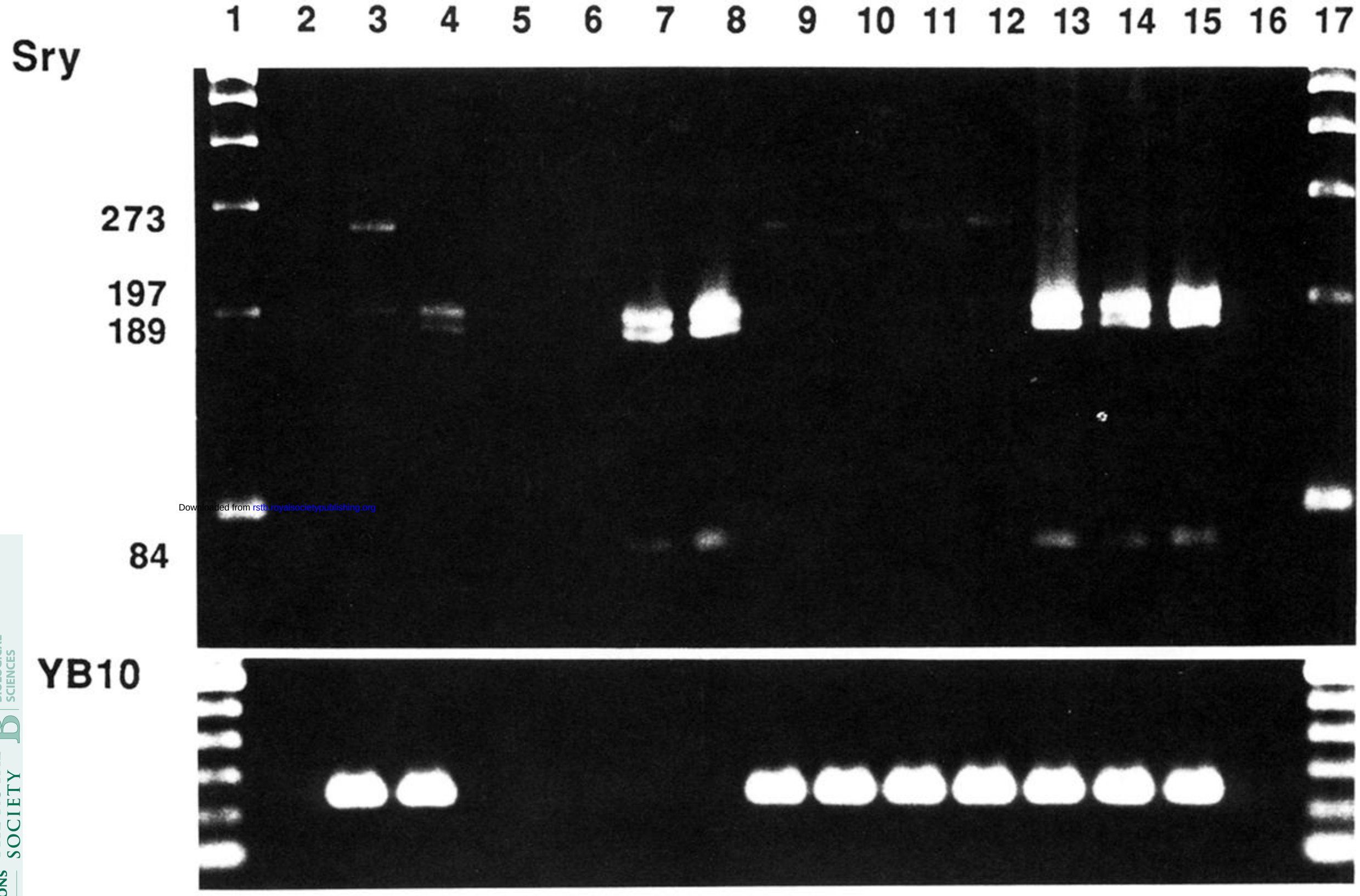
M. A. Ferguson-Smith (*Department of Pathology, Cambridge University, U.K.*). Can one infer from Dr Eicher's findings that the three autosomal loci that Dr Eicher has mapped, contain the target sequence for the DNA binding region of *Sry*?

E. M. Eicher. If the autosomal genes we have mapped are activated or inactivated by *Sry*, these genes would be expected to contain the target sequences for the DNA binding region of *Sry*. However, if these genes are involved in activating *Sry* or they are transcription factors involved in activating genes in the ovarian pathway, they would be expected to contain a different DNA binding sequence. Because the *Sry* target sequence is relatively common in the genome, even finding this sequence in one of these genes does not guarantee that it is regulated by *Sry*. Until the autosomal genes are cloned, we can only speculate on their function and one can argue either side of the coin.

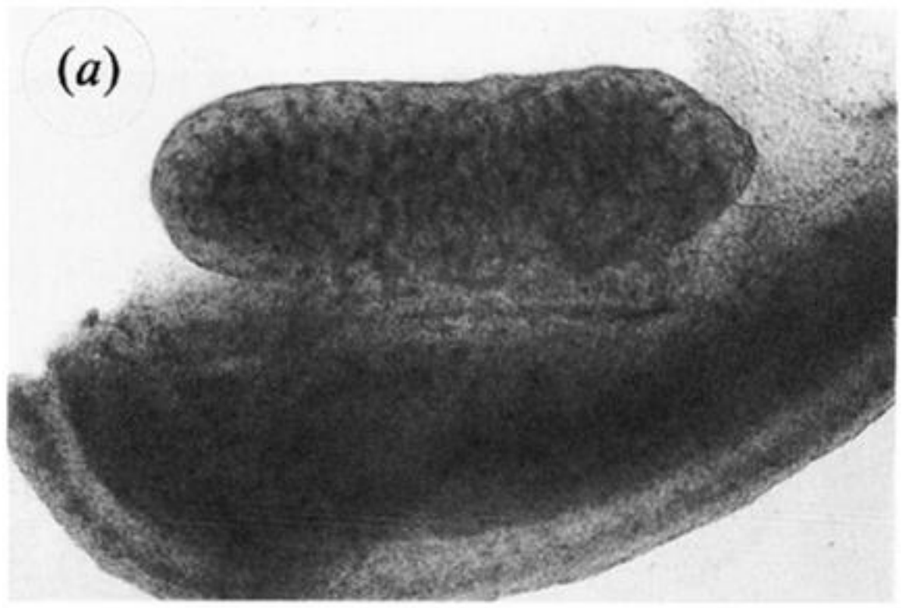
U. Mittwoch (*Department of Anatomy, Queen Mary & Westfield College, London, U.K.*). If it is a requirement for the testicular pathway to occur before the ovarian pathway, it would seem necessary for the first gene in the testicular pathway to accelerate testicular development. It might also be helpful if genes in the ovarian pathway would ensure slow gonadal development.

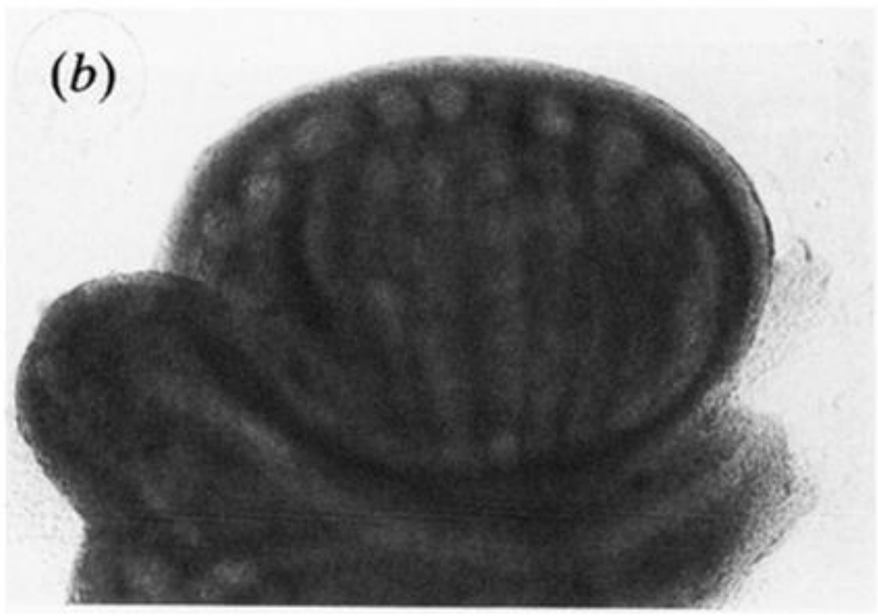
E. M. Eicher. Clearly one of the genes involved in the development of the total testis causes rapid growth of testicular tissue. It does not therefore follow that a gene in the ovarian pathway ensures slow growth of the foetal ovary.

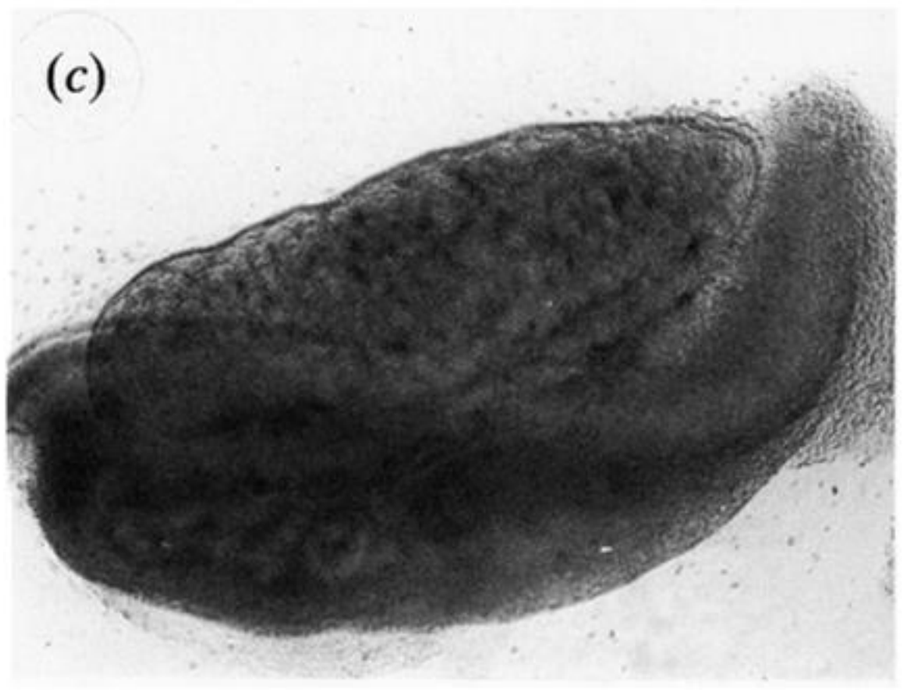
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

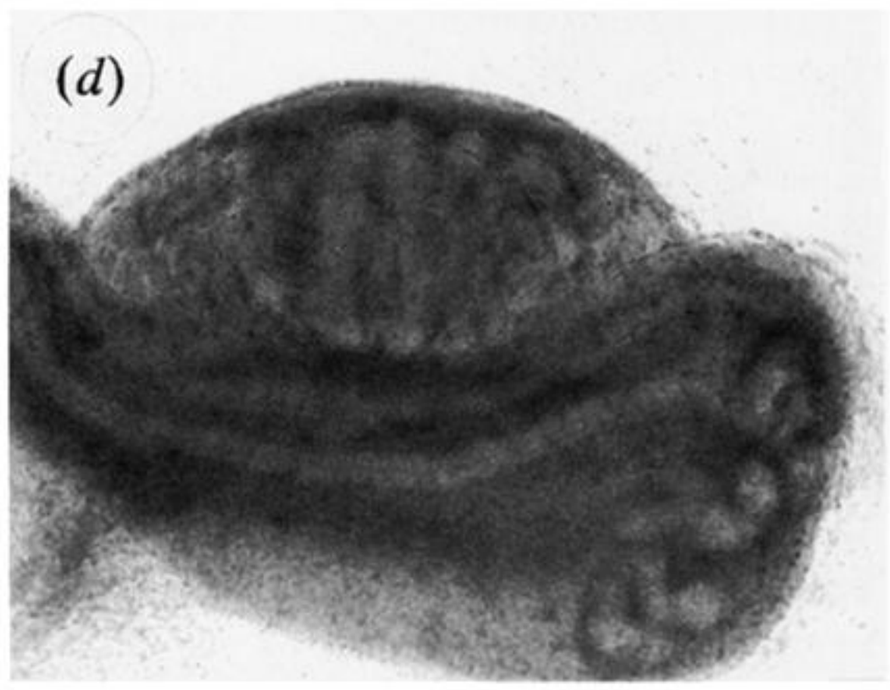


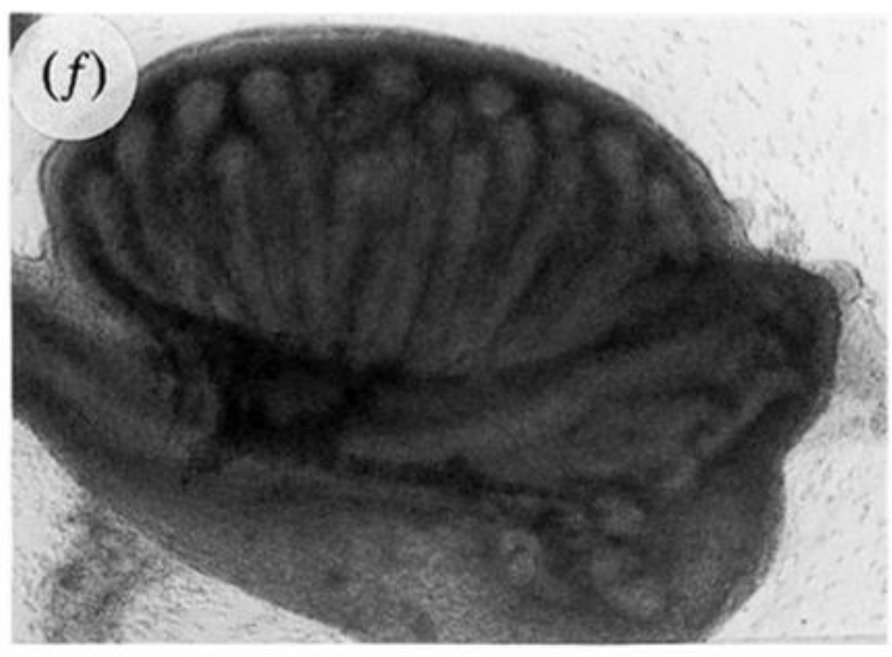
gure 2. Determination of the presence of *Tg4* and the Y chromosome. Top, presence of the transgene was determined / PCR amplification of a 470 b.p. segment from the *Sry* gene using the primer pair: 5′-AGATCTTGATTTTTA-TGTTC-3′; 5′-GAGTACAGGTGTGCAGCTCTA-3′) (Gubbay *et al.* 1990). PCR products were cut with *Mbo*I, ıd the resulting fragments separated by size on a 4% NuSieve 3:1 agarose gel. Two fragments (273 and 197 b.p.) 'e diagnostic for Sry^{POS} and three fragments (197, 189, and 84 b.p.) are diagnostic for Sry^{129} (the 129 PCR product ontains an additional *Mbo*I site). Lanes 1–4, 16 and 17 represent control DNA samples: 1 and 17 are molecular size arkers, 16 is a no DNA control, 2 is a C57BL/6J XX female, 3 is a C57BL/6J XY^{POS} hermaphrodite, and 4 is a 29/J male. Lanes 5–15 represent DNA samples from C57BL/6J fetuses from the *Tg4* experiment: 5 and 6 are XX males, 7 and 8 are XX *Tg4* males, lane 9 and 10 are XY^{POS} hermaphrodites, 11 and 12 are XY^{POS} females, and 13, 4, and 15 are XY^{POS} *Tg4* males. Bottom, the higher copy number of the *Tg4* transgene made it difficult to determine resence of the endogenous Sry^{POS} gene (i.e. the *M. d. poschiavinus* Y chromosome). To circumvent this problem, the resence of the Y^{POS} chromosome was verified by PCR amplification using the primer pair: YB10L, 5′-CCCTTTCTAAGCAGACATC-3′; and YB10R 5′-TCACACTAACCTCACAGGCC-3′. These primers were erived from a Y chromosome sequence, designated YB10, that is present in 300–500 copies on the long arm of the ouse Y chromosome (Eicher *et al.* 1989; Tucker *et al.* 1989; Tucker *et al.* 1992). The YB10 primers amplify a 225 b.p. agment from male DNA. Under some conditions, PCR products are noted in female DNA; their sizes, however, differ om the fragment obtained from male DNA. DNA samples are the same as in (*a*) above.

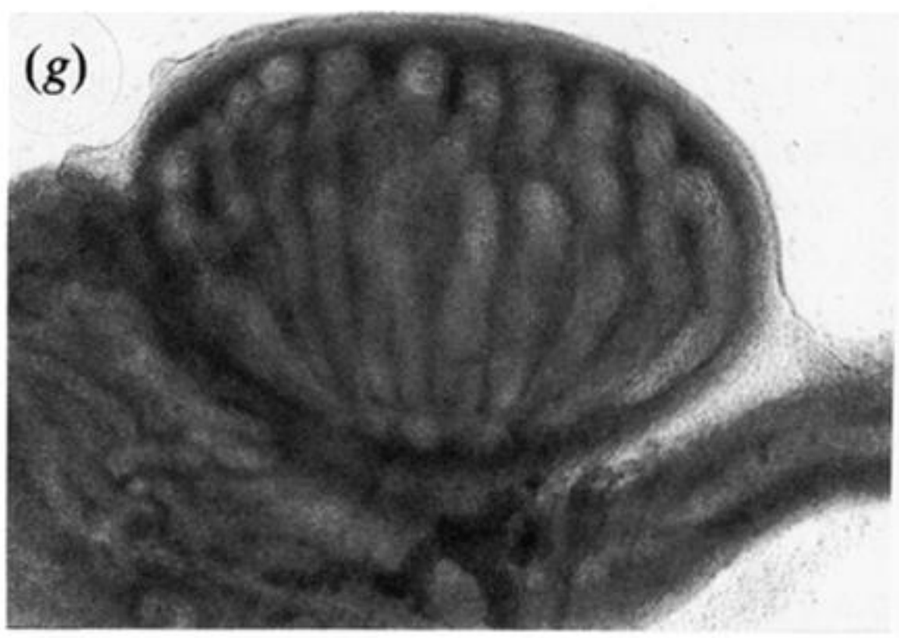












igure 3. Gonads with attached mesonephric complexes from fetuses at 14.5 days of development. Gonads (*a*) and) represent an ovary from a normal C57BL/6J XX female and a testis from a normal C57BL/6J XY male, spectively. Gonad (*c*) is an ovary from a C57BL/6J XY^{POS} female and gonad (*d*) is an ovotestis from a C57BL/6J Y^{POS} hermaphrodite. In the ovotestis, note the presence of testicular tissue in the central region with ovarian tissue both ends. Gonads (*e*) and (*f*) are the paired testes from a C57BL/6J XX *Tg4* male and gonads (*g*) and (*h*) are e paired testes from a C57BL/6J XY^{POS} *Tg4* individual.